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March 17, 2003

The Honorable Christine Whitman
Administrator (1101A)
U.S. Environmental Protection Agency
1200 Pennsylvania Ave., NW
Washington, DC 20460

Re: Comments on Ameribrom's HPV Test Plan for 2,2-bis(bromomethyl)-1,3-propandiol (BBMP-diol) Materials Category

Dear Administrator Whitman:

The following comments on the Ameribrom Incorporated's High Production Volume (HPV) Challenge test plan for 2,2-bis(bromomethyl)-1,3-propandiol (BBMP-diol) are submitted on behalf of People for the Ethical Treatment of Animals, the Physicians Committee for Responsible Medicine, the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These animal, health, and environmental protection organizations have a combined membership of more than ten million Americans.

Ameribrom's test plan for BBMP-diol is for a single chemical used as a fire retardant in a variety of products. The description of the chemical's structure, use, properties, and existing animal test data are clear and concise. However, we remain very concerned about the remaining proposed testing on animals, which consists of the following:

1. An acute toxicity study in fish (OECD No. 203)

2. A developmental toxicity study (OECD No. 414)

These tests are unnecessary. If this test plan is conducted in its present form, approximately 960 animals will be killed. Our objections are summarized as follows:

Similar to our comments on more than 30 previous test plans in the HPV program that called for acute fish toxicity testing, we urge Ameribrom to use alternatives to the acute fish study, such as ECOSAR, TETRATOX, or the recently validated *Dar*T Test. The high K_{ow} of this compound points to this being an ideal substance for such an analysis. TETRATOX, an assay based on the protozoan *Tetrahymena pyriformis* (Larsen, 1997), is an appropriate method for use in this plan. With 50% growth impairment as the endpoint, the results of this assay show close similarity to toxicity in the fathead minnow (Schultz, 1997). On October 23, 2001, PETA and PCRM held a meeting with the EPA to review and facilitate incorporation of an *in vitro* aquatic toxicity test into the HPV program, and Dr. Schultz (Professor of Predictive Toxicology, University of Tennessee College of Veterinary Medicine) made a presentation about TETRATOX. The extensive available information demonstrates that TETRATOX is a high quality alternative to fish testing. It is in fact already used extensively in industry, and is being considered as a candidate OECD test guideline.

The recently validated *Dar*T Test (Nagel 2002) is another prospective replacement for *in vivo* studies. The test protocol and performance parameters are described in detail in Schulte and Nagel (1994) and Nagel (1998). Briefly, however, the *Dar*T test uses fertilized zebrafish (*Danio rerio*) eggs as a surrogate for living fish; because the eggs will not hatch during the test period, the *Dar*T is classified as a non-animal test. The exposure period is 48-hours, and assessed endpoints include coagulation, development of blastula, gastrulation, termination of gastrulation, development of somites, movements, extension of the tail, development of eyes, heartbeat, circulation, heart rate, pigmentation, and edema. Endpoints comparable to lethality *in vivo* include failure to complete gastrulation after 12-hours, no somites after 16-hours, no heartbeat after 48-hours, and coagrulated eggs. The other endpoints provide further insight for a more detailed assessment of the effects of test substances. The reliability and relevance of the *Dar*T test have recently been confirmed through an international, multi-laboratory validation study coordinated and financed by the German Environmental Protection Agency; predictions of acute toxicity from the *Dar*T test were highly concordant with *in vivo* reference data (Schulte *et al.* 1996). This *in vitro* test has been accepted in Germany as a replacement for the use of fish in the assessment of wastewater effluent (Friccius *et al.*1995), and is clearly suitable for immediate use as a replacement to the use of fish in the HPV program's screening-level toxicity studies.

Ameribrom's proposal to conduct the OECD 414 for developmental toxicity cannot be supported for the following reasons:

1. Existing animal data point to this compound being a genotoxic carcinogen, a reproductive hazard, and an overall chronic hazard. Conducting another study focused simply on the developmental toxicity of this compound will provide no further useful information that will effect the regulation and control of this chemical. Logically, if a chemical is a reproductive hazard, whether or not it is a developmental hazard becomes largely immaterial. Even more importantly, developmental toxicity is not an endpoint in and of itself in the HPV program. Give the fact that a multigenerational reproductive toxicity test has already been conducted and the mice were not affected in terms of survival and growth (though the number of live pups per litter was reduced), and necropsy of mice pups from this group showed no developmentally-related effects other than reduced birth weight, further developmental testing cannot be supported. This existing study clearly indicates that further testing will simply show that developmental effects are secondary to reproductive effects and points to the uselessness of further developmental testing of this compound.

   Ameribrom needs to adhere to the principles set forth in the October 1999 letter to HPV program participants regarding the use of thoughtful toxicology, is particular the requirement that "before generating new information, participants should further consider whether any additional information obtained would be useful or relevant."

2. An *in vivo* study using 900 rabbits in stressful experiments is simply unjustified. As another alternative to *in vivo* testing, and given the aforementioned reasons why such a test is unwarranted, an *in vitro* embryotoxicity test would be adequate to characterize any possible adverse embryotoxic effects of this material. If, in fact, Ameribrom insists on further exploration of developmental endpoints, we urge it to consider the use of an *in vitro* test for embryotoxicity (a critical endpoint in developmental toxicity) using the rodent Embryonic

Stem Cell Test (EST) protocol that has been validated by the European Centre for the Validation of Alternative Methods (ECVAM; Genschow *et al.* 2002). If a positive result is found, the substance should be treated as a developmental toxicant/teratogen, and no further testing should be conducted under the screening-level HPV program. Although we have written to the EPA repeatedly concerning the inclusion of the embryonic stem cell test in the HPV program, with correspondence dating back more than six months, we have received no reply. We urge Ameribrom to correspond directly with the EPA on the incorporation of this validated non-animal test.

3. While it is inappropriate for Ameribrom to conduct any mammalian developmental toxicity testing, it is appalling that this company is proposing to conduct the OECD test guideline 414, which kills approximately 900 rabbits. The December 26, 2000 *Federal Register* notice on the "voluntary" HPV program, entitled, "Data Collection and Development on High Production Volume Chemicals," specifically states that the OECD 422 should be used (which kills 675 animals) or that a rationale should be provided if the 422 is not to be used. However, as stated in item no. 1 above, given the fact that a multigenerational reproduction study has already been carried out on this substance, no further testing is warranted in this screening program.

For all the above reasons, we urge Ameribrom to drop any further toxicity testing on animals. I can be reached at 757-622-7382, ext. 1304, or via e-mail at JessicaS@peta.org should you wish to discuss these issues further.

Sincerely,

Jessica Sandler, MHS
Federal Agency Liaison

Literature Cited

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